

CLAIMS

1. A method for determining the presence or amount of testosterone in a test sample comprising,

(a) ionizing testosterone from said test sample to produce one or more testosterone ions detectable by mass spectrometry having a mass/charge ratio selected from the group consisting of 289.1 ± 0.5 , 109.2 ± 0.5 , and 96.9 ± 0.5 ; and

(b) detecting the presence or amount of the testosterone ion(s) by mass spectrometry, wherein the presence or amount of the testosterone ion(s) is related to the presence or amount of testosterone in the test sample.

2. The method of claim 1 wherein the ionizing of step (a) comprises, ionizing said testosterone from said sample to provide a precursor ion having a mass/charge ratio (m/z) of about 289.1 ± 0.5 ;

isolating the precursor ion by mass spectroscopy; and effecting a collision between the isolated precursor ion and an inert collision gas to produce one or more testosterone ions detectable by mass spectrometry having a mass/charge ratio selected from the group consisting of 109.2 ± 0.5 , and 96.9 ± 0.5 .

3. The method of claim 1 wherein testosterone from the test sample is purified prior to step (a)

4. The method of claim 3 wherein testosterone from the test sample is purified by high turbulence liquid chromatography (HTLC).

5. The method of claim 4, wherein the purifying step comprises:

- (i) applying the sample to an HTLC extraction column;
- (ii) washing the HTLC extraction column under conditions whereby testosterone is retained by the column;
- (iii) eluting retained testosterone from the HTLC extraction column;
- (iv) applying the retained material to an analytical column; and
- (v) eluting purified testosterone from the analytical column.

6. The method of claim 5 wherein the HTLC extraction column is a C-12 extraction column, and the analytical column is a C-18 analytical column.

7. The method of claim 6 wherein the HTLC extraction column comprises particles of about 50 μm , and the C-18 analytical column comprises particles of about 4 μm .

8. The method of claim 5, wherein steps (i)-(v) are performed in an in-line automated fashion.

9. The method of claim 8, wherein steps (a) and (b) are performed in an in-line automated fashion with steps (i)-(v).

10. The method of claim 1 wherein the ionization in step (a) is performed by a method selected from the group consisting of electrospray ionization, chemical ionization, photon ionization, matrix-assisted laser desorption ionization (MALDI), and surface enhanced laser desorption ionization (SELDI).

11. The method of claim 1 wherein the ionization in step (a) is performed by atmospheric pressure photoionization (APPI).

12. The method of claim 11 wherein the testosterone is ionized by electrospray ionization.

13. The method of claim 11 wherein the testosterone ion is in a gaseous state.

14. The method of claim 2 wherein the inert collision gas is selected from the group consisting of: argon and nitrogen.

15. The method of claim 13 wherein the collision gas is nitrogen.

16. The method of claim 13 wherein the collision gas is argon.

17. The method of claim 1 wherein the test sample is obtained from a human.

18. The method of claim 1 wherein the test sample is blood, serum, plasma, or urine.

19. The method of claim 17 wherein the test sample is deproteinated prior to the ionization step.

20. The method of claim 18 wherein the deproteination is performed by exposing the test sample to formic acid.

21. The method of claim 1 wherein the mass spectroscopy is MS/MS/TOF mass spectroscopy.

22. The method of claim 1 wherein the mass spectroscopy is MALDI/MS/MS/TOF mass spectroscopy.
23. The method of claim 1 wherein the mass spectroscopy is SELDI/MS/MS/TOF mass spectroscopy.
24. The method of claim 1 wherein the mass spectroscopy is APPI/MS/MS/TOF mass spectroscopy.
25. The method of claim 1 wherein the presence or amount of the testosterone ion is related to the presence or amount of testosterone in the test sample by comparison to a reference sample.
26. The method of claim 25, wherein the reference sample is 2, 2, 4, 6, 6-d₅ testosterone.
27. The method of claim 26, wherein said reference sample is detected by ionizing said reference to produce one or more ions detectable by mass spectrometry having a mass/charge ratio selected from the group consisting of 294.1 ± 0.5 , 113.2 ± 0.5 , and 99.9 ± 0.5 .
28. The method of claim 27 wherein said ionizing comprises generating a precursor ion having a mass/charge ratio (m/z) of about 294.1 ± 0.5 ;
isolating the precursor ion by mass spectroscopy; and
effecting a collision between the isolated precursor ion and an inert collision gas to produce one or more daughter ions detectable by mass spectrometry having a mass/charge ratio selected from the group consisting of 113.2 ± 0.5 , and 99.9 ± 0.5 .
29. The method of claim 1 further comprising determining the specificity of testosterone detection by calculating a ratio of the daughter ions for the sample and comparing that ratio with a daughter ion ratio for a purified sample of testosterone.
30. The method of claim 1 wherein detecting the presence or amount of the testosterone ion(s) by mass spectrometry involves combining the signal for ion 109.2 ± 0.5 with the signal for ion 96.9 ± 0.5 to obtain a summed daughter ion signal.
31. The method of claim 30 wherein the amount of testosterone is detected by comparing the summed daughter ion signal with a standard curve of summed daughter ion signals for known amounts of testosterone.

32. The method of claim 27 wherein detecting the presence or amount of the reference testosterone ion(s) by mass spectrometry involves combining the signal for ion 113.2 ± 0.5 , with the signal for ion and 99.9 ± 0.5 to obtained a summed daughter ion signal.

33. The method of claim 2 wherein the mass spectroscopy is MS/MS/TOF mass spectroscopy.

33. The method of claim 2 wherein the mass spectroscopy is MALDI/MS/MS/TOF mass spectroscopy.

34. The method of claim 2 wherein the mass spectroscopy is SELDI/MS/MS/TOF mass spectroscopy.

35. The method of claim 2 wherein the mass spectroscopy is APPI/MS/MS/TOF mass spectroscopy.

36. The method of claim 2 wherein the presence or amount of the testosterone ion is related to the presence or amount of testosterone in the test sample by comparison to a reference sample.

37. The method of claim 36, wherein the reference sample is 2, 2, 4, 6, 6-d₅ testosterone.

38. The method of claim 37, wherein said reference sample is detected by ionizing said reference to produce one or more ions detectable by mass spectrometry having a mass/charge ratio selected from the group consisting of 294.1 ± 0.5 , 113.2 ± 0.5 , and 99.9 ± 0.5 .

39. The method of claim 38 wherein said ionizing comprises generating a precursor ion having a mass/charge ratio (m/z) of about 294.1 ± 0.5 ;

isolating the precursor ion by mass spectroscopy; and

effecting a collision between the isolated precursor ion and an inert collision gas to produce one or more daughter ions detectable by mass spectrometry having a mass/charge ratio selected from the group consisting of 113.2 ± 0.5 , and 99.9 ± 0.5 .

40. The method of claim 2 further comprising determining the specificity of testosterone detection by calculating a ratio of the daughter ions for the sample and comparing that ratio with a daughter ion ratio for a purified sample of testosterone.

41. The method of claim 2 wherein detecting the presence or amount of the testosterone ion(s) by mass spectrometry involves combining the signal for ion 109.2 ± 0.5 with the signal for ion 96.9 ± 0.5 to obtained a summed daughter ion signal.

42. The method of claim 41 wherein the amount of testosterone is detected by comparing the summed daughter ion signal with a standard curve of summed daughter ion signals for known amounts of testosterone.

43. The method of claim 38 wherein detecting the presence or amount of the reference testosterone ion(s) by mass spectrometry involves combining the signal for ion 113.2 ± 0.5 , with the signal for ion and 99.9 ± 0.5 to obtained a summed daughter ion signal.